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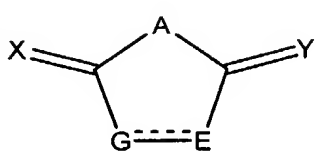
(54) Title: HETEROCYCLIC INHIBITORS OF GLYCOGEN SYNTHASE KINASE GSK-3



(I)

(57) Abstract: Compounds of formula (I) where A, E, G, X, Y, and the bond --- take various meanings are of use in the preparation of a pharmaceutical formulation, for example in the treatment of a disease in which GSK-3 is involved, including Alzheimer's disease or the non-dependent insulin diabetes mellitus, or hyperproliferative disease such as cancer, displasias or metaplasias of tissue, psoriasis, arteroscleroses or restenosis.

HETEROCYCLIC INHIBITORS OF GLYCOGEN SYNTHASE KINASE GSK-3

Field of the Invention

The present invention relates to enzyme inhibitors, and more particularly to heterocyclic inhibitors of glycogen synthase kinase 3β, GSK-3.

Background of the Invention

Alzheimer's disease (AD) is a neurodegenerative process characterised by cognitive disorders associated with a progressive deterioration of the cholinergic function, and neuropathological lesions as senile plaques, formed by the fibrillary β-amyloid, and neurofibrillary tangles, bundles of paired helical filaments.

Generally speaking, AD is restricted to groups aged 60 years or more and is the most common cause of dementia in the elderly population. Today, AD affects 23 million people worldwide. As longevity increases, it is estimated that by the year 2050 the number of cases of AD will more than triplicate [Amaduci, L.; Fratiglioni, L. "Epidemiology of AD: Impact on the treatment", in *Alzheimer Disease: Therapeutic Strategies,* E. Giacobini and R. Becker, Eds., Birhäuser, EEUU, 1994, pp. 8].

Two major histological lesions are observed in AD brains associated with the neuronal loss: neurofibrillary tangles and senile plaques at the intracellular and extracellular level respectively ["Alzheimer Disease: From molecular biology to therapy", E. Giacobini and R. Becker, Eds., Birhäuser, EEUU, 1996].

Neurofibrillary tangles are structures formed by paired helical filaments (PHFs). They are comprised mainly of the microtubule-associated protein (MAP) tau in an abnormally hyperphosphorylated state [Grundke-Iqbal, I.; Iqbal, K.; Tung, Y.C.; Quinlan, M.;

Wisniewski, H.M.; Binder, L.I., "Abnormal phosphorylation of the microtubule-associated protein tau in Alzheimer cytoskeletal pathology", *Proc. Natl. Acad. Sci. USA*, 1986, *83*, 4913-4917; Grundke-Iqbal, I.; Iqbal, K.; Quinlan, M.; Tung, Y.C.; Zaidi, M.S.; Wisniewski, H.M., "Microtubule-associated protein tau. A component of the Alzheimer paired helical filaments", *J. Biol. Chem.*, 1986, *261*, 6084-6089; Greenberg, S.G.; Davies, P.; Schein, J.D.; Binder, L.I., "Hydrofluoric acid-treated tau PHF proteins display the same biochemical properties as normal tau.", *J. Biol. Chem.*, 1992, *267*, 564-569]. Such aberrant phosphorylation of tau, determined by the effects of different protein kinases and phosphatases, appears to compromise on its ability to bind to and stabilise microtubules and this may contributes to AD pathology [Moreno, F.J.; Medina, M.; Perez, M.; Montejo de Garcini, E.; Avila, J., "Glycogen sintase kinase 3 phosphorylation of different residues in the presence of different factors: Analysis on tau protein", *FEBS Lett.*, 1995, *372*, 65-68]. Thus, the blockade of this hyperphosphorylation step may be a prime target at which to interrupt the pathogenic cascade. The selective inhibitors of tau kinases might be new effective drugs for the treatment of AD.

The search for tau kinases inhibitors is a field of a great interest. Tau can be phosphorylated by several proline-directed protein kinases (PDKs) and non-PDKs. However, in AD the exact role of any of these kinases in the abnormal hyperphosphorylation of tau is not yet understood and to date, the activity of these kinases has not been found to be upregulated. It is no doubt that glycogen synthase kinase 3β (GSK-3β) is an *in vivo* tau kinase in the brain [Lovestone, S.; Hartley, C.L.; Pearce, J.; Anderton, B.H., "Phosphorylation of tau by glycogen synthase-3 in intact mammalian cells: the effects on the organization and stability of microtubules", *Neuroscience*, 1996, *73*, 1145-1157; Wagner, U.; Utton, M.; Gallo, J.M.; Miller, C.C., "Cellular phosphorylation of tau by GSK-3β influences tau binding to microtubules and microtubule organisation", *J. Cell. Sci.*, 1996, *109*, 1537-1543; Ledesma, M.; Moreno, F.J.; Perez, M.M.; Avila, J., "Binding of apolipoprotein E3 to tau protein: effects on tau glycation, tau phosphorylation and tau-microtubule binding, in vitro", *Alzheimer Res.*, 1996, *2*, 85-88]. These findings open the gate to the use of GSK-3β inhibitors as therapeutical agents in the treatment of AD. At the moment few compounds are known with this enzymatic inhibitory property.

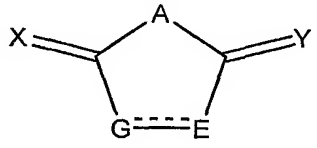
Lithium behaves as a specific inhibitor of the GSK-3 family of protein kinases *in vitro* and in intact cells Muñoz-Montaño, J.R.; Moreno, F.J.; Avila, J.; Diaz-Nido, J., "Lithium inhibits Alzheimer's disease-like tau protein phosphorylation in neurons", *FEBS Lett.*, 1997, *411,* 183-188].

Finally, it is observed that insulin inactivates GSK-3 and it is shown that the non-dependent insulin diabetes mellitus is developed with the activation of this enzyme. So that, GSK-3 inhibitors would be a new therapy for the non-dependent insulin diabetes mellitus.

In our work team we have recently discovered a new family of small synthetic heterocyclic molecules with GSK-3β inhibitory properties at micromolar level.

Description of the Invention

The invention is directed to the compounds represented by the general formula I:



(I)

where:

A is $-C(R^1)_2-$, -O- or $-NR^1-$;

E is $-NR^1-$ or $-CR^1R^2-$ and the substituent R^2 is absent if - - - is a second bond between E and G;

G is -S-, $-NR^1-$ or $-CR^1R^2-$ and the substituent R^2 is absent if - - - is a second bond between E and G;

- - - may be a second bond between E and G where the nature of E and G permits and E with G optionally then forms a fused aryl group;

R^1 and R^2 are independently selected from hydrogen, alkyl, cycloalkyl, haloalkyl, aryl, $-(Z)_n$-aryl, heteroaryl, $-OR^3$, $-C(O)R^3$, $-C(O)OR^3$, $-(Z)_n-C(O)OR^3$ and $-S(O)_t-$ or as indicated R^2 can be such that E with G then form a fused aryl group;

Z is independently selected from $-C(R^3)(R^4)-$, $-C(O)-$, $-O-$, $-C(=NR^3)-$, $-S(O)_t-$, $N(R^3)-$;

n is zero, one or two;

t is zero, one or two;

R^3 and R^4 are independently selected from hydrogen, alkyl, aryl and heterocyclic; and

X and Y are independently selected from $=O$, $=S$, $=N(R^3)$ and $=C(R^1)(R^2)$.

Detailed Description of the Invention

As used in this specification and appended claims, unless specified to the contrary, the following terms have the meaning indicated:

"Alkyl" refers to a straight or branched hydrocarbon chain radical consisting of carbon and hydrogen atoms, containing no saturation, having one to eight carbon atoms, and which is attached to the rest of the molecule by a single bond, e.g., methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, n-pentyl, etc. Alkyl radicals may be optionally substituted by one or more substituents independently selected from the group consisting of a halo, hydroxy, alkoxy, carboxy, cyano, carbonyl, acyl, alkoxycarbonyl, amino, nitro, mercapto and alkylthio.

"Alkoxy" refers to a radical of the formula $-OR_a$ where R_a is an alkyl radical as defined above, e.g., methoxy, ethoxy, propoxy, etc.

"Alkoxycarbonyl" refers to a radical of the formula $-C(O)OR_a$ where R_a is an alkyl radical as defined above, e.g., methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, etc.

"Alkylthio" refers to a radical of the formula $-SR_a$ where R_a is an alkyl radical as defined above, e.g., methylthio, ethylthio, propylthio, etc.

"Amino" refers to a radical of the formula $-NH_2$.

"Aryl" refers to a phenyl or naphthyl radical, preferably a phenyl radical. The aryl radical may be optionally substituted by one or more substituents selected from the group consisting of hydroxy, mercapto, halo, alkyl, phenyl, alkoxy,

haloalkyl, nitro, cyano, dialkylamino, aminoalkyl, acyl and alkoxycarbonyl, as defined herein.

"Aralkyl" refers to an aryl group linked to an alkyl group. Preferred examples include benzyl and phenethyl.

"Acyl" refers to a radical of the formula -C(O)-R_c and -C(O)-R_d where R_c is an alkyl radical as defined above and R_d is an aryl radical as defined above, e.g., acetyl, propionyl, benzoyl, and the like.

"Aroylalkyl" refers to an alkyl group substituted with -C(O)-R_d. Preferred examples include benzoylmethyl.

"Carboxy" refers to a radical of the formula -C(O)OH.

"Cyano" refers to a radical of the formula -CN

"Cycloalkyl" refers to a stable 3- to 10-membered monocyclic or bicyclic radical which is saturated or partially saturated, and which consist solely of carbon and hydrogen atoms. Unless otherwise stated specifically in the specification, the term "cycloalkyl" is meant to include cycloalkyl radicals which are optionally substituted by one or more substituents independently selected from the group consisting of alkyl, halo, hydroxy, amino, cyano, nitro, alkoxy, carboxy and alkoxycarbonyl.

"Fused aryl" refers to an aryl group, especially a phenyl or heteroaryl group, fused to the five-membered ring.

"Halo" refers to bromo, chloro, iodo or fluoro.

"Haloalkyl" refers to an alkyl radical, as defined above, that is substituted by one or more halo radicals, as defined above, e.g., trifluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 1-fluoromethyl-2-fluoroethyl, and the like.

"Heterocycle" refers to a heterocyclyl radical. The heterocycle refers to a stable 3- to 15-membered ring which consists of carbon atoms and from one to five heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur, preferably a 4- to 8- membered ring with one or more heteroatoms, more preferably a 5- or 6- membered ring with one or more heteroatoms. For the purposes of this invention, the heterocycle may be a monocyclic, bicyclic or tricyclic ring system, which may include fused ring systems; and the nitrogen,

carbon or sulfur atoms in the heterocyclyl radical may be optionally oxidised; the nitrogen atom may be optionally quaternized; and the heterocyclyl radical may be partially or fully saturated or aromatic. Examples of such heterocycles include, but are not limited to, azepines, benzimidazole, benzothiazole, furan, isothiazole, imidazole, indole, piperidine, piperazine, purine, quinoline, thiadiazole, tetrahydrofuran. The hetrocycle may be optionally substituted by R^3 and R^4 as defined above in the summary of the invention.

"Heteroaryl" refers to an aromatic heterocycle

"Mercapto" refers to a radical of the formula -SH

"Nitro" refers to a radical of the formula $-NO_2$

The invention is in particular directed to the enzymatic activity against kinases of the compounds of the general formula I.

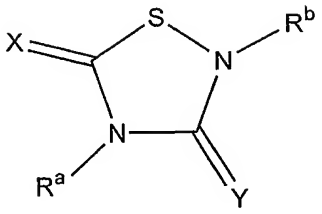
A is preferably selected from $-C(R^1)_2-$ and $-NR^1-$.

Preferably R^1 is selected from hydrogen, alkyl, cycloalkyl, aryl (optionally substituted with a group selected from alkyl, halo and alkoxy), $-C(R^3)(R^4)$-aryl (the aryl part being optionally substituted with a group selected from alkyl, halo and alkoxy), $-OR^3$, $-C(O)OR^3$ and
$-C(R^3)(R^4)-C(O)OR^3$, and R^3 and R^4 are independently selected from hydrogen and alkyl.

The subscript n is preferably zero or one, and n will be chosen having regard to the known chemistry of possible groupings.

X and Y are preferably oxygen or sulphur, at least one of X and Y is preferably oxygen.

A particularly preferred class of compounds is of the formula (II).



(II)

where R^a and R^b are independently selected from hydrogen, alkyl, cycloalkyl, haloalkyl, aryl,

$-(Z)_n$-aryl, heteroaryl, $-OR^3$, $-C(O)R^3$, $-C(O)OR^3$,$-(Z)_n-C(O)OR^3$ and $-S(O)t-$, and

Z, n, t, R^3, R^4, X and Y are as defined above.

In the formula (II), X and Y are preferably selected from oxygen, sulphur, and $-NR^3-$ where R^3 is heterocyclic, especially a 6-membered heterocycle which has one heteroatom which is nitrogen, being optionally aromatic and optionally oxidised or quaternised. More preferably, both X and Y are both oxygen.

Preferably. R^a and R^b are independently selected from hydrogen, alkyl, cycloalkyl, aryl (optionally substituted with a group selected from alkyl, halo and alkoxy), $-C(R^3)(R^4)$-aryl (the aryl part being optionally substituted with a group selected from alkyl, halo and alkoxy),

$-OR^3$, $-C(O)OR^3$ and $-C(R^3)(R^4)-C(O)OR^3$, and R^3 and R^4 are independently selected from hydrogen, alkyl and heterocyclic.

More preferably R^a and R^b are independently selected from alkyl, aryl (optionally substituted with a group selected from alkyl, halo and alkoxy), $-CH_2$-aryl (the aryl part being optionally substituted with a group selected from alkyl, halo and alkoxy), and $-CH_2-C(O)OR^3$ where R^3 is hydrogen or alkyl.

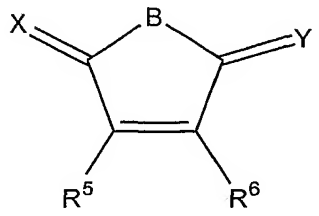
Still more preferably, R^a and R^b are independently selected from methyl, ethyl, propyl, benzyl, phenyl (optionally substituted with a group selected from methyl, fluoro, chloro, bromo and methoxy) and $-CH_2$-C(O)O-ethyl.

The most preferred compounds of formula (II) are listed in Table 1 below.

Table 1

R^a	R^b	X	Y
CH_2Ph	Me	O	O
Et	Me	O	O
Ph	Me	O	O
CH_2CO_2Et	Me	O	O
4-OMePh	Me	O	O
4-MePh	Me	O	O
4-BrPh	Me	O	O
4-FPh	Me	O	O
4-ClPh	Me	O	O
CH_2Ph	CH_2Ph	O	S
Ph	Ph	O	S

Another preferred class of compounds of the invention are those compounds of formula (III):



(III)

wherein:

B is $-NR^7-$ or $C(R^7)(R^8)-$ (wherein R^7 and R^8 are independently selected from hydrogen, alkyl, aryl, $-CH_2-W-aryl$, and $-W-CO_2H$, and W is a single bond, CH_2 or CO);

R^5 and R^6 are independently selected from hydrogen, alkyl, aryl and $-CH_2-aryl$; and

X and Y are independently selected from =O and =S.

In the formula (III), B is preferably $-NR^7-$, wherein R^7 is selected from hydrogen, alkyl and $-CH_2-aryl$, especially hydrogen, methyl or benzyl.

R^5 and R^6 are preferably hydrogen.

X and Y are preferably oxygen.

The most preferred compounds of formula (III) are listed in Table 2 below.

Table 2

B	X	Y	R^5	R^6
NH	O	O	H	H
$N-CH_2Ph$	O	O	H	H
NMe	O	O	H	H
CH_2	O	O	H	H

Examples of further classes of compounds of formula I include those where:

a) A is $-CH_2-$; E is $-CR^1R^2-$, preferably $-CH_2-$; G is $-CR^1R^2-$, preferably $-CH_2-$;

b) A is $-CH_2-$; E is $-CR^1-$, preferably -CH-; G is $-CR^1-$, preferably -CH-; and - - - - is a second bond between G and E;

c) A is -O-; E is $-CR^1-$, preferably -CH-; G is $-CR^1-$, preferably -CH-; and - - - - is a second bond between G and E;

d) A is $-NR^1-$, where R^1 is preferably hydrogen, alkyl or aralkyl; E is $-CR^1-$, preferably -CH-; G is $-CR^1-$, preferably -CH-; and - - - - is a second bond between G and E;

e) A is $-NR^1-$, where R^1 is preferably hydrogen or aralkyl; E is $-CR^1R^2-$, preferably $-CH_2-$; G is $-CR^1R^2-$, preferably $-CH_2-$;

f) A is $-NR^1-$, where R^1 is preferably hydrogen or aralkyl; E is $-CR^1-$; G is $-CR^1-$; - - - - is a second bond between E and G; and E with G form a fused aryl group, preferably a phenyl group;

g) A is $-NR^1-$, where R^1 is preferably hydrogen, alkyl, carboxyalkyl, aroylalkyl or aralkyl; E is -S; G is $-C(R^1)_2-$, preferably $-CH_2-$;

h) A is $-NR^1$, where R^1 is preferably aryl; E is $-NR^1-$, where R^1 is preferably hydrogen or alkyl; G is $-NR^1-$, where R^1 is preferably hydrogen or alkyl.

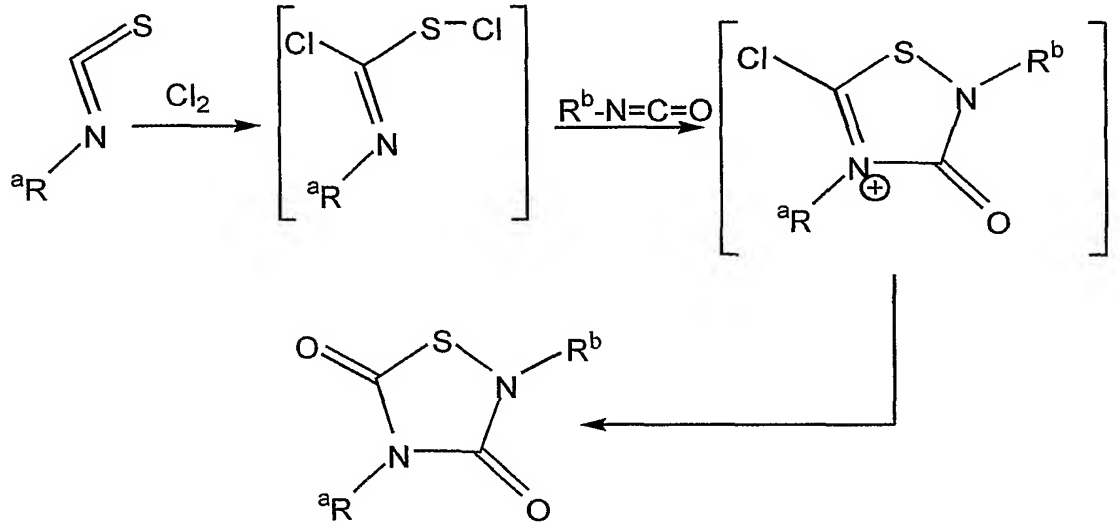
In these classes of compounds, X and Y are preferably both O, though for class (g) X can be O and Y can be S. When E with G form a fused phenyl group, the resultant compounds are phthalimido derivatives.

Synthesis of the Compounds of the Invention:

The compounds of the invention can be synthesised by available procedures.

For preferred compounds of formula (II) a general procedure is available [Martinez, A.; Castro, A.; Cardelús, I.; Llenas, J.; Palacios, J.M. *Bioorg. Med. Chem.*, 1997, *5*, 1275-1283].

Concretely, the compounds of general formula (II) and collected in Table I, were prepared following the synthetic procedure depicted in scheme 1, and using the reactivity of N-alkyl-S-[N'-chlorocarbamoyl)amino]isothiocarbamoyl chlorides with different alkyl isocyanates. The isothiocyanates chlorination is performed by addition of an equimolecular quantity of chlorine over an hexane solution of the mentioned isothiocyanate at -15 ºC. The reaction of the iminochloroalkylsulfenyl chloride formed with alkyl or arylisocyanate under inert atmosphere and subsequent hydrolysis, yielded the thiadiazolidindiones described in table I.



Scheme 1

The typical compounds of this invention selectively inhibit GSK-3β without inhibition of others protein kinases such as PKA, PKC, CK-2 and CdK2, which could eliminate the widespread effects. GSK-3β is involved in the aetiopathogenesis of AD and it is responsible for the abnormal hyperphosphorylation of the tau protein. The selective inhibitors here disclosed can be useful therapeutical agents for the treatment of neurodegeneratives diseases associated to the pathology of tau protein, specially for AD which forms part of this invention. The inhibitory action of these compounds against

GSK-3β leads for the design of drugs able to stop the formation of the neurofibrilar tangles, one of the hallmark present in this neurodegenerative process.

These compounds can be useful for the treatment of other pathologies in which the GSK-3β is involved, such as non-insulin-dependent diabetes mellitus.

Additionally, these compounds can be useful for the treatment of hyperproliferative diseases such as displasias and metaplasias of different tissues, psoriasis, artherioschlerosis, resthenosis and cancer, due to their inhibition of cellular cycle which forms part of this invention.

Accordingly, the present invention further provides pharmaceutical compositions comprising a compound of this invention together with a pharmaceutically acceptable carrier or diluent. Appropriate dosage forms and dosing rates can be devised and adopted in accordance with conventional practice.

Examples

Example 1.- Enzymatic inhibition of the compounds of the invention

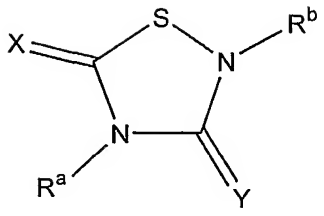
GSK-3β inhibition: The GSK-3 activity was determined by incubation of a mixture of GSK-3 enzyme (Sigma), a phosphate source and a GSK-3 substrate in the presence and in the absence of the corresponding test compound, and by measuring the GSK-3 activity of this mixture.

Concretely, the GSK-3 activity is determined by incubating the enzyme at 37 °C during 20 minutes in a final volume of 12 µl of buffer (50 mM tris, pH = 7.5, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 10 mM Cl_2Mg) supplemented with 15 µM (final concentration) of the synthetic peptide GS 1 [Woodgett, J. R. "Use of peptides for affinity purification of protein-serine kinases", *Anal. Biochem.*, 1989, *180*, 237-241] as substrate, 15 µM of ATP, 0.2 μC_i of [γ-^{32}P]ATP and different concentrations of the test compound. The reaction is quenched by addition of an aliquot of the reaction mixture in phosphocelullose p81 papers.

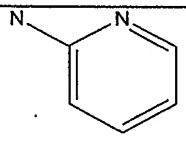
These papers are washed three times with phosphoric acid 1% and the radioactivity incorporated to the GS 1 peptide is measured in a liquid scintillation counter

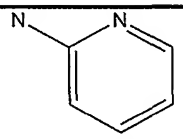
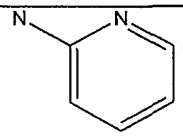
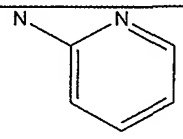
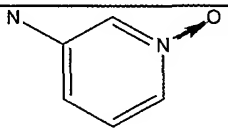
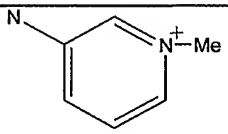
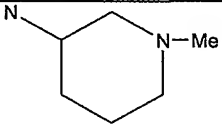
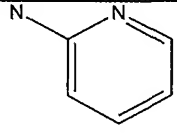
Compounds showed in table I are representative of the GSK-3 inhibitory activity object of this invention. The IC_{50} (concentration at which a 50% of enzyme inhibition is shown) values are gathered in Table 3 below.

Table 3



(II)

R^a	R^b	X	Y	IC_{50} (μM)
CH_2Ph	Me	O	O	1
Et	Me	O	O	5
Et	nPr	O	O	10
Et	cyclohexyl	O	O	10
Ph	Me	O	O	2
CH_2CO_2Et	Me	O	O	5
4-OMePh	Me	O	O	5
CH_2Ph	Et	O	O	7
Et	iPr	O	O	35
CH_2Ph	Et	O	S	6
CH_2Ph	CH_2Ph	O	S	10
Ph	Ph	O	S	20
Et	Et	O	S	20
Cyclohexyl	Me	O	O	>100
4-MePh	Me	O	O	5
4-BrPh	Me	O	O	3
4-FPh	Me	O	O	4
4-ClPh	Me	O	O	4
Et	Me		O	>100

Et	Et		O	>100
Et	H		O	>100
Me	Me		O	>100
Et	Me		O	>100
Et	Me		O	>100
Et	Me		O	>100
Et	Me		S	10

GSK-3 inhibition: The experiments of inhibition were also performed at variable concentrations of ATP (up to 50 μM) and in all cases the same value of IC_{50} were obtained. Thus could suggest that thiadiazolindiones do not compete with ATP in the binding to GSK-3.

The first four compounds were assayed for inhibition of other enzymes.

Protein kinase A (PKA) inhibition: The potential inhibition of this enzyme is evaluated by determining the esthatmine phosphorylation by the protein kinase A (PKA). The esthatmine was purified following the procedure described by Belmont and Mitchinson

(Belmont, L. D.; Mitchinson, T. J. "Identification of a protein that interact with tubulin dimers and increases the catastrophe rate of microtubule", *Cell*, 1996, *84*, 623-631).

Concretely, it was used purified PKA (Sigma, catalytic subunit from bovine heart (p 2645)) and 10-15 μg of substrate (esthatmine) in a 25 μl total volume of buffer solution containing 20 μM (γ-^{32}P)ATP. The cAMP kinase protein (100 ng/reaction) was performed in 50 μl of 25 mM hepes, pH 7.4, 20 mM $MgCl_2$, 2 mM EGTA, 2 mM dithiothreitol, 0.5 mM Na_3VO_4. After the reaction took place, a quenching buffer was added, the reaction mixture was boiled at 100 oC during 5 minutes and the phosphorylated protein was characterized by gel electrophoresys and quantified by autoradiographia.

In these conditions none of the compounds assayed showed any inhibition of PKA.

Protein kinase C (PKC) inhibition: The potential inhibition of this enzyme is evaluated by determining the phosphorylation of the peptide PANKTPPKSPGEPAK (Woodgett, J. R. "Use of peptides for affinity purification of protein-serine kinases", *Anal. Biochem.*, 1989, *180*, 237-241) by the protein kinase C (PKC) using phosphatidyl serine as stimulating agent. The method followed is the same described above for GSK-3.

Concretely, it was used PKC purified from rat brains following the method described by Walsh (Walsh, M. P.; Valentine, K. A.; Nagi, P. K.; Corruthers, C. A.; Hollenberg, M. D. *Biochem. J.*, 1984, *224*, 117-127) and 1-10 mM of substrate in a total volume of 25 μl of adecuated buffer solution containing 10 μM (γ-^{32}P)ATP.

In these conditions none of the compounds assayed showed any inhibition of PKC.

Casein kinase 2 (CK-2) inhibition: The phosphorylating activity of this enzyme against esthatmine has been measured using CK-2 purified from bovine brains, following the method described by Alcazar (Alcazar, A.; Marín, E.; Lopez-Fando, J.; Salina, M. "An improved purification procedure and properties of casein kinase II from brain", *Neurochem. Res.*, 1988, *13*, 829-836), with 3.6 μM of substrate in a total volume of 25 μl

of an adequate buffer solution containing 20 μM (γ-^{32}P)ATP. The CK-2 assays were performed with esthatmine as substrate (see PKA determination) in 50 μl of 25 mM Hepes, pH 7.4, 20 mM $MgCl_2$, 2 mM EGTA, 2 mM dithiothreitol, 0.5 mM Na_3VO_4, and 100 ng of purified CK-2. After the reaction took place, it was followed the same method described for PKA.

In these conditions none of the compounds assayed showed any inhibition of CK-2.

Cyclin dependent protein kinase 2 (Cdc2) inhibition: The phosphorylating activity of this enzyme against histone H1 has been measured using Cdc2 (Calbiochem) following the method described by Kobayashi (Kobayashi, H.; Stewart, E.; Poon, R.Y.; Hunt, T. "Cyclin A and cyclin B dissociate from p34cdc2 with half-times of 4 and 15 h, respectively, regardless of the phase of the cell cycle", *J. Biol. Chem.*, 1994, *269*, 29153-29160), with 1 μg/μl of substrate in a total volume of 25 μl of the adequate buffer solution containing 20 μM (γ-^{32}P)ATP. The Cdc2 assays were performed with histone H1 as substrate (see PKA determination) in 50 μl of buffer pH 7.5, 50 mM Tris-HCl, 10 mM Cl_2Mg, 1 mM DTT, 1 mM EGTA, 100 μM ATP, 0.01% BRIJ-35. After the reaction took place, it was followed the same method described for PKA.

In these conditions none of the compounds assayed showed any inhibition of Cdc2.

Example 2.- Analysis of the neurites growth after the drug treatment.

Cells were maintained in a Dulbecco medium (DEMEM) with a 10 % fethal bovine serum, glutamine (2 mM) and antibiotics. For the analysis of the potential GSK-3 inhibition in vivo, mice neuroblastoms N_2A cultures (Garcia-Perez, J.; Avila, J.; Diaz-Nido, J. "Lithium induces morphological differentiation of mouse neuroblastoma", *J. Neurol. Res.*, 1999, *57*, 261-270) were used. The test compounds were added to these cells cultures. This cell line has the particularity of expressed a certain kind of neuronal phenotype (neuritic extensions) after the addition of lithium chloride (10 mM), a known GSK-3 inhibitor. After 2-3 days of culture, it was check the effect of the tested compounds gathered in table I. It was observed the generation of neuritic extension in the same extension than when

lithium was added. That fact confirms the in vivo GSK-3 inhibition of the compounds of the invention.

Example 3.- Cell cycle blockade.

In parallel, the potential interference of these compounds with the cell cycle was studied on N_2A cells. The cell culture was maintained in a Dulbecco medium (DEMEM) with a 10 % fethal bovine serum, glutamine (2 mM) and antibiotics.

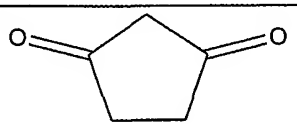
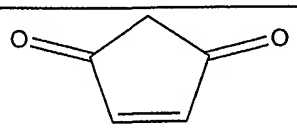
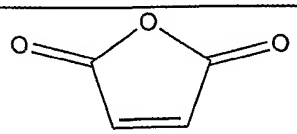
The first four compounds of general formula (I) gathered in Table 3 were assayed in the described conditions and shown ability to inhibit the cell cycle at an inhibitor concentration comprised between 100 nM and 1μM. The cellular blockade was initially observed at concentrations comprised between 100-200 nM and was totally effective at 1 μM.

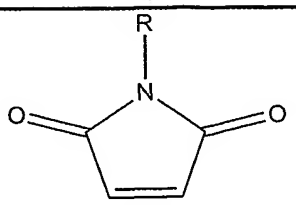
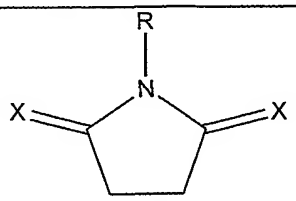
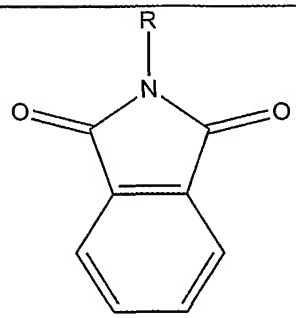
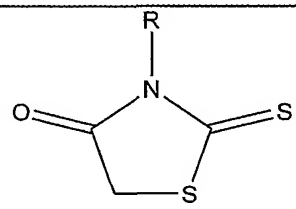
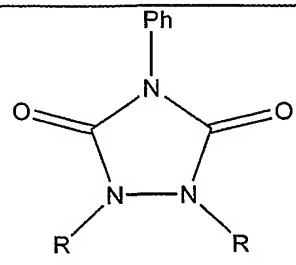
The tested compounds was non toxic in stationary fibroblast culture MRC-5 after 10 days of continue exposure to the inhibitors.

Example 4. - GSK-3 inhibition of further compounds

GSK-3 inhibition data

Table 4

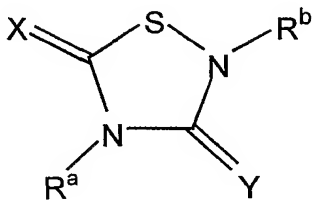
Family			IC_{50} (μM)
a			>100
b			12
c			

d		R=H R=CH_2Ph R=Me	6 1 5
e		R=H; X, Y=O R=CH_2Ph; X, Y=O R=CH_2Ph; X=O; Y=H	>100 >100 >100
f		R=H R=CH_2Ph	>100 >100
g		R=H R=Me R=CH_2CO_2H R=CH_2Ph R=CH_2CH_2Ph R=CH_2COPh	>100 >100 >100 25 35 50
h		R=H R=Me	>100 >100

GSK-3 inhibitors: For compounds belong to family D, the experiments of GSK-3 inhibition were also performed at variable concentrations of ATP (up to 50 μM) and in all cases the same value of IC_{50} were obtained. Thus could suggest that these compounds do not compete with ATP in the binding to GSK-3.

Example 5.- Cell cycle blockade

The IC_{50} for some of the compounds tested in N_2A cell cultures are gathered in Table 5 below.



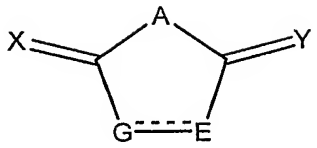
(II)

Table 5

R^a	R^b	X	Y	IC_{50} (μM)
CH_2Ph	Me	O	O	4-8
Et	Me	O	O	40-100
Et	nPr	O	O	5-10
Et	cyclohexyl	O	O	6-9
Ph	Me	O	O	4-7
CH_2CO_2Et	Me	O	O	1-2
4-OMePh	Me	O	O	1-2
CH_2Ph	Et	O	O	4-7
CH_2Ph	CH_2Ph	O	O	2-3
Et	Et	O	O	30-80
CH_2Ph	CH_2Ph	O	S	1-2
Ph	Ph	O	S	4-8

Claims

1. Compounds of general formula (I):



(I)

wherein:

A is $-C(R^1)_2-$, -O- or $-NR^1-$;

E is $-NR^1-$ or $-CR^1R^2-$ and the substituent R^2 is absent if - - - is a second bond between E and G;

G is -S-, $-NR^1-$ or $-CR^1R^2-$ and the substituent R^2 is absent if - - - is a second bond between E and G;

- - - may be a second bond between E and G where the nature of E and G permits and E with G optionally then forms a fused aryl group;

R^1 and R^2 are independently selected from hydrogen, alkyl, cycloalkyl, haloalkyl, aryl, $-(Z)_n$-aryl, heteroaryl, $-OR^3$, $-C(O)R^3$, $-C(O)OR^3$, $-(Z)_n-C(O)OR^3$ and $-S(O)_t-$ or as indicated R^2 can be such that E with G then form a fused aryl group;

Z is independently selected from $-C(R^3)(R^4)-$, -C(O)-, -O-, $-C(=NR^3)-$, $-S(O)_t-$ and $N(R^3)-$;

n is zero, one or two;

t is zero, one or two;

R^3 and R^4 are independently selected from hydrogen, alkyl, aryl and heterocyclic; and

X and Y are independently selected from =O, =S, $=N(R^3)$ and $=C(R^1)(R^2)$.

2. A compound according to claim 1, wherein A is selected from $-C(R^1)_2-$ and $-NR^1-$, and R^1 is defined in claim 1.

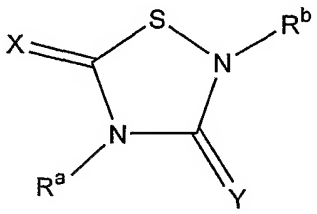
3. A compound according to claim 1, wherein R^1 and R^2 are independently selected from hydrogen, alkyl, cycloalkyl, aryl (optionally substituted with a group selected from alkyl, halo and alkoxy), $-C(R^3)(R^4)$-aryl (the aryl part being optionally substituted with a group

selected from alkyl, halo and alkoxy), $-OR^3$, $-C(O)OR^3$ and $-C(R^3)(R^4)-C(O)OR^3$, and R^3 and R^4 are independently selected from hydrogen and alkyl.

4. A compound according to claim 1, wherein X and Y are independently selected from =O and =S.

5. A compound according to claim 1, wherein:
A is selected from $-C(R^1)_2-$ and $-NR^1-$, and
R^1 is selected from hydrogen, alkyl, cycloalkyl, aryl (optionally substituted with a group selected from alkyl, halo and alkoxy), $-C(R^3)(R^4)$-aryl (the aryl part being optionally substituted with a group selected from alkyl, halo and alkoxy), $-OR^3$, $-C(O)OR^3$ and $-C(R^3)(R^4)-C(O)OR^3$, and R^3 and R^4 are independently selected from hydrogen and alkyl; and
X and Y are independently selected from =O and =S.

6. A compound according to claim 1, having the general formula (II):



(II)

wherein:
R^a and R^b are independently selected from hydrogen, alkyl, cycloalkyl, haloalkyl, aryl, $-(Z)_n$-aryl, heteroaryl, $-OR^3$, $-C(O)R^3$, $-C(O)OR^3$,$-(Z)_n-C(O)OR^3$ and $-S(O)_t-$, and
Z, n, t, R^3, R^4, X and Y are as defined in claim 1.

7. A compound according to claim 6, wherein R^a and R^b are independently selected from hydrogen, alkyl, cycloalkyl, aryl (optionally substituted with a group selected from alkyl, halo and alkoxy), $-C(R^3)(R^4)$-aryl (the aryl part being optionally substituted with a group

selected from alkyl, halo and alkoxy), $-OR^3$, $-C(O)OR^3$ and $-C(R^3)(R^4)-C(O)OR^3$, and R^3 and R^4 are independently selected from hydrogen, alkyl and heterocyclic.

8. A compound according to claim 7, wherein R^a and R^b are independently selected from alkyl, aryl (optionally substituted with a group selected from alkyl, halo and alkoxy), $-CH_2-$aryl (the aryl part being optionally substituted with a group selected from alkyl, halo and alkoxy), and $-CH_2-C(O)OR^3$, and R^3 is hydrogen or alkyl.

9. A compound according to claim 8, wherein R^a and R^b are independently selected from methyl, ethyl, propyl, benzyl, phenyl (optionally substituted with a group selected from methyl, fluoro, chloro, bromo and methoxy) and $-CH_2-C(O)O$-ethyl.

10. A compound according to claim 6, wherein X and Y are independently selected from =O, =S and $=NR^3$ (wherein R^3 is heterocyclic).

11. A compound according to claim 10, wherein X is =O.

12. A compound according to claim 11, wherein X is =O and Y is =O.

13. A compound according to claim 6, wherein:
R^a and R^b are independently selected from hydrogen, alkyl, cycloalkyl, aryl (optionally substituted with a group selected from alkyl, halo and alkoxy), $-C(R^3)(R^4)$-aryl (the aryl part being optionally substituted with a group selected from alkyl, halo and alkoxy), $-OR^3$, $-C(O)OR^3$ and $-C(R^3)(R^4)-C(O)OR^3$,
R^3 and R^4 are independently selected from hydrogen, alkyl and heterocyclic, and
X and Y are independently selected from =O, =S and $=NR^3$.

14. A compound according to claim 13, wherein:
R^a and R^b are independently selected from hydrogen, alkyl, cycloalkyl, aryl (optionally substituted with a group selected from alkyl, halo and alkoxy), $-C(R^3)(R^4)$-aryl (the aryl

part being optionally substituted with a group selected from alkyl, halo and alkoxy), $-OR^3$, $-C(O)OR^3$ and $-C(R^3)(R^4)-C(O)OR^3$,

R^3 and R^4 are independently selected from hydrogen and alkyl; and

X is =O.

15. A compound according to claim 14, wherein:

R^a and R^b are independently selected from methyl, ethyl, propyl, benzyl, phenyl (optionally substituted with a group selected from methyl, fluoro, chloro, bromo and methoxy) and $-CH_2-C(O)O$-ethyl;

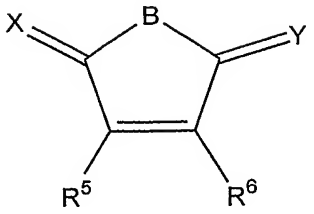
X is =O; and

Y is =O.

16. The following compounds according to claim 6:

R^a	R^b	X	Y
CH_2Ph	Me	O	O
Et	Me	O	O
Ph	Me	O	O
CH_2CO_2Et	Me	O	O
4-OMePh	Me	O	O
4-MePh	Me	O	O
4-BrPh	Me	O	O
4-FPh	Me	O	O
4-ClPh	Me	O	O
CH_2Ph	CH_2Ph	O	S
Ph	Ph	O	S

17. A compound according to claim 1, having the general formula (III):



(III)

wherein:

B is $-NR^7-$ or $C(R^7)(R^8)-$ (wherein R^7 and R^8 are independently selected from hydrogen, alkyl, aryl, $-CH_2-W-aryl$, and $-W-CO_2H$, and W is a single bond, CH_2 or CO);

R^5 and R^6 are independently selected from hydrogen, alkyl, aryl and $-CH_2-aryl$; and

X and Y are independently selected from =O and =S.

18. A compound according to claim 17, wherein B is $-NR^7-$, and R^7 is selected from hydrogen, alkyl and $-CH_2-aryl$.

19. A compound according to claim 18, wherein B is $-NR^7-$, and R^7 is hydrogen, methyl or benzyl.

20. A compound according to claim 17, wherein R^5 and R^6 are hydrogen.

21. A compound according to claim 17, wherein X is =O and Y is =O.

22. The following compounds according to claim 17:

B	X	Y	R^5	R^6
NH	O	O	H	H
$N-CH_2Ph$	O	O	H	H
NMe	O	O	H	H
CH_2	O	O	H	H

23. A pharmaceutical formulation containing as active ingredient a compound as defined in any of claims 1 to 22.

24. Use of a compound as defined in any of claims 1 to 22 in the preparation of a pharmaceutical formulation.

25. Use according to claim 24, where the pharmaceutical formulation is for the treatment of a disease in which GSK-3 is involved, including Alzheimer's disease or non-insulin dependent diabetes mellitus.

26. Use according to claim 24 where the pharmaceutical formulation is for the treatment of a hyperproliferative disease such as cancer, displasias or metaplasias of tissue, psoriasis, arteriosclerosis or restenosis.

27. A method for the treatment of a disease in which GSK-3 is involved, comprising administering to a human in need of such treatment an effective amount of a compound according to any one of claims 1 to 22.

28. A method according to claim 27, where the disease is selected from Alzheimer's disease and non- insulin dependent diabetes mellitus.

29. A method according to claim 27, where the disease is a hyperproliferative disease such as cancer, displasias or metaplasias of tissue, psoriasis, arteriosclerosis or restenosis.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/02100

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D207/44 C07D285/08 C07D209/48 C07C49/597 A61K31/433
A61P25/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 21927 A (FENWICK ASHLEY EDWARD ;HOLDER JULIE CAROLINE (GB); SMITH DAVID GLY) 20 April 2000 (2000-04-20) page 102 -page 103; claims 18-21; examples D1,D19	1-25,27
X	WO 95 17182 A (LILLY CO ELI) 29 June 1995 (1995-06-29) page 21, column 2 -page 21, column 28; claim 1	1-25,27
A	EP 0 711 773 A (SUNTORY LTD) 15 May 1996 (1996-05-15) page 2, line 42 - line 48	1-25,27

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search	Date of mailing of the international search report
28 August 2001	25/09/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Timmermans, M

INTERNATIONAL SEARCH REPORT

national Application No
PCT/GB 01/02100

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 38675 A (HOLDER JULIE CAROLINE ;SMITH DAVID GLYNN (GB); COGHLAN MATTHEW PAU) 6 July 2000 (2000-07-06) page 5, paragraph 5; examples A,B,C	1-25,27
P,A	WO 01 09106 A (SMITH DAVID GLYNN ;WARD ROBERT WILLIAM (GB); SMITHKLINE BEECHAM PL) 8 February 2001 (2001-02-08) the whole document	1-25,27

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-25(in part),26,27(in part),28-29

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible either to provide the Applicant with the said documents neither to determine which parts of the claims may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, a meaningful search over the whole breadth of the claims is impossible. Consequently, the search has been restricted to the compounds corresponding to formula (II) of claim 6 or formula (III) of claim 17 and having the desired pharmacological properties of claims 25 and 27 (inhibiting glycogen synthase kinase GSK-3 or protein kinases or useful against Alzheimer's disease).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 01/02100

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